

**T84 Cells | 300354****General information**

<b>Description</b>	This line exhibits tight junctions, and desmosomes between adjacent cells. The cells should be maintained at high density (at least 1/4 confluency).
<b>Organism</b>	Human
<b>Tissue</b>	Colon
<b>Disease</b>	Carcinoma
<b>Metastatic site</b>	Lung
<b>Applications</b>	Colorectal cancer research; intestinal epithelial biology; tight junction and barrier function studies; colonic transport physiology; cystic fibrosis transmembrane conductance regulator (CFTR) research; drug absorption and metabolism; xenograft models
<b>Synonyms</b>	T-84, T 84

**Characteristics**

<b>Age</b>	72 years
<b>Gender</b>	Male
<b>Ethnicity</b>	Ethnicity unspecified
<b>Morphology</b>	Epithelial-like
<b>Cell type</b>	Epithelial cells
<b>Growth properties</b>	Adherent

**Regulatory Data**

<b>Citation</b>	T84 (Cytion catalog number 300354)
<b>Biosafety level</b>	1
<b>NCBI_TaxID</b>	9606

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**CellosaurusAccession** CVCL\_0555

**GMO Status** No genetic modification; wildtype colon carcinoma cell line (KRAS G13D heterozygous mutation is an endogenous somatic change, not a genetic engineering modification)

### Biomolecular Data

**Receptors expressed** Peptide hormone, neurotransmitter

**Antigen expression** Keratin + (Immunoperoxidase staining)

**Isoenzymes** G6PD, B, PGM1, 1, PGM3, 1, ES-D, 1, Me-2, 1-2, AK-1, 1, GLO-1, 1-2

**Tumorigenic** Yes, in nude mice

**Products** Carcinoembryonic antigen (CEA), 600 ng/ml per 10 exp6 cells per 10 days, keratin

**Mutational profile** T84 cells carry a heterozygous Kras mutation in codon13: GGC(Wt Gly) >GAC(Asp)

**Karyotype** The stemline modal chromosome number is 56, occurring at 28% with polyploidy at 12.4%. Eighteen markers are common to most metaphases examined. Normal x and chromosome 13 were absent, chromosomes 2, 4 and 22 were single-copied, and chromosome 12 was 4-copied.No Y chromosome was detected by Q band observation. DM occurred in nearly 50% of the cells.

### Handling

**Culture Medium** Ham's F12, w: 1.0 mM stable Glutamine, w: 1.0 mM Sodium pyruvate, w: 1.1 g/L NaHCO<sub>3</sub> (Cytion article number 820600a)

**Supplements** Supplement the medium with 10% FBS

**Dissociation Reagent** Accutase

**Doubling time** approx. 48 to 72 hours

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**Subculturing** Remove the old medium from the adherent cells and wash them with PBS that lacks calcium and magnesium. For T25 flasks, use 3-5 ml of PBS, and for T75 flasks, use 5-10 ml. Then, cover the cells completely with Accutase, using 1-2 ml for T25 flasks and 2.5 ml for T75 flasks. Let the cells incubate at room temperature for 8-10 minutes to detach them. After incubation, gently mix the cells with 10 ml of medium to resuspend them, then centrifuge at 300xg for 3 minutes. Discard the supernatant, resuspend the cells in fresh medium, and transfer them into new flasks that already contain fresh medium.

**Split ratio** 1 to 3

**Seeding density** 1 to  $2 \times 10^4$  cells/cm<sup>2</sup> (maintain at minimum 1/4 confluency to preserve tight junction phenotype)

**Fluid renewal** 2 times per week

**Post-Thaw Recovery** After thawing, plate the cells at  $5 \times 10^4$  cells/cm<sup>2</sup> and allow at least 24–48 hours for adherence. Maintain cells at high density ( $\geq 25\%$  confluency) to preserve tight junction formation.

**Freeze medium** As a cryopreservation medium, we use complete growth medium (including FBS) + 10% DMSO for adequate post-thaw viability, or CM-1 (Cytion catalog number 800100), which includes optimized osmoprotectants and metabolic stabilizers to enhance recovery and reduce cryo-induced stress.

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### Thawing and Culturing Cells

1. Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit.
2. Upon receipt, either store the cryovial immediately at temperatures below  $-150^{\circ}\text{C}$  to ensure the preservation of cellular integrity, or proceed to step 3 if immediate culturing is required.
3. For immediate culturing, swiftly thaw the vial by immersing it in a  $37^{\circ}\text{C}$  water bath with clean water and an antimicrobial agent, agitating gently for 40-60 seconds until a small ice clump remains.
4. Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
5. Carefully open the disinfected vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of room-temperature culture medium, mixing gently.
6. Centrifuge the mixture at  $300 \times g$  for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
7. Gently resuspend the cell pellet in 10 ml of fresh culture medium. For adherent cells, divide the suspension between two T25 culture flasks; for suspension cultures, transfer all the medium into one T25 flask to promote effective cell interaction and growth.
8. Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

### Incubation Atmosphere

$37^{\circ}\text{C}$ , 5%  $\text{CO}_2$ , humidified atmosphere.

### Shipping Conditions

Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately  $-78^{\circ}\text{C}$  throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage.

### Storage Conditions

For long-term preservation, place vials in vapor-phase liquid nitrogen at about  $-150$  to  $-196^{\circ}\text{C}$ . Storage at  $-80^{\circ}\text{C}$  is acceptable only as a short interim step before transfer to liquid nitrogen.

## Quality Control & Molecular Analysis

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**Sterility**

Mycoplasma contamination is excluded using both PCR-based assays and luminescence-based mycoplasma detection methods.

To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections.